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(71) Applicant: AMYLIN PHARMACEUTICALS, INC.
San Diego California 92121 (US)

(72) Inventor: Cooper, Garth James Smith
Woodstock, Oxon OX7 1SR (GB)

(74) Representative: Baillie, Iain Cameron
Langner Parry
52-54 High Holborn
London WC1V 6RR (GB)

Remarks:
This application was filed on 12 - 12 - 1996 as a divisional application to the application mentioned under INID code 62.

(54) Amylin isolated from pancreatic islet amyloid and pharmaceutical compositions comprising it

(57) A synthetic peptide is provided having the amino acid sequence:

KCNTATCATQRLANFLVHSSNNFGAILSSTNVG SNTY

said peptide being carboxy terminally amidated and having a disulphide bond between the cysteine residues at positions 2 and 7. This is useful in treatment of diabetes mellitus. There is also provided a synthetic carboxy terminally amidated peptide which is a conservative variant of the above peptide. The peptide biological activity can be enhanced by refolding the peptide in a denaturing solution. If the peptide is produced in the form of pharmaceutical compositions, it can be obtained from the islet amyloid of diabetic pancreata by solubilising and successively subjecting to normal phase high performance liquid chromatography gel filtration and reverse phase high performance liquid chromatography followed by elution.

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Description

This invention relates to peptides, fragments thereof and preparation of compositions therefrom.

Type 1 diabetes mellitus is a disease that affects large numbers of people, and results from the destruction of B-cells within the islets of Langerhans in the pancreas. The current therapy for type 1 diabetes is with parenteral administration of replacement doses of insulin. It is desirable that diabetic control be such that blood glucose levels be returned to near normal in order to avoid the long term complications of diabetes. Such therapy is, however, difficult to control in that it is frequently not easy to avoid the complication of hypoglycaemia, which may lead to morbidity, hypoglycaemic coma, and in infrequent cases to long term brain damage or death. It has long been known that, for reasons which are not fully understood, hypoglycaemia is a very frequent and very upsetting side-effect of insulin therapy.

Type 2 diabetes mellitus is about 8 to 10 times more prevalent than Type 1 diabetes, and may affect up to 4% of the adult population in Western countries. It is characterized by (1) a deficiency but not an absolute lack of insulin secretion which results in hypoglycaemia, and usually also by (2) varying degrees of resistance to the actions of insulin. In this form of diabetes, unlike Type 1, B-cells are retained in the islets in normal or only slightly reduced numbers. Islet amyloid is also found in most cases (Clark A., Cooper G.J.S. et al, Lancet August 2, 1987).

British Patent Application 8709871 filed 27 April 1987, describes a novel peptide which is identical to or substantially homologous with the amino acid sequence:

```
    5    10   15   20   25   30   35
    |    |    |    |    |    |    |
KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY
```

or an active subfragment thereof. Cooper et al., "Purification and Characterization of a Peptide from Amyloid-Rich Pancreases of Type 2 Diabetic Patients," Proc. Natl. Acad. Sci. USA.- Vol. 84 (1987).

This may alternatively be written using the classical three letter designations of amino acid residues as follows:

```
1               5                   10                  15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe

16              20                  25                  30
Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr

31              35
Asn Val Gly Ser Asn Thr Tyr
```

This novel peptide, provisionally named diabetes associated peptide or DAP, and now renamed "amylin", has been isolated and characterized from the amyloid containing pancreases of type 2 diabetic humans. It is stated that amylin may be found to have clinical utility, such as appetite suppressant activity, and perhaps also vasodilator activity which could be either general activity or be specific for pancreas or islet blood flow.

The above peptide contains thirty-seven amino acid residues and is structurally similar to calcitonin gene related peptide CGRP, having 46% identity with human CGRP-2. The following table compares the primary structure of 1) the above peptide with that of 2) human CGRP-2, 3) human CGRP-1 and 4) rat CGRP-1. Amino acid identity between peptides is indicated by boxes. Dotted boxes indicate area of displaced homology.

```
   1       5         10        15        20        25        30        35
1) K C N T A T C A T Q R L A N F L V H S S N N F G A I L S S T N V G S N T Y
2) A C N T A T C V T H R L A G L L S R S G G H V K S N F V P T N V G S K A F
3) A C D T A T C V T H R L A G L L S R S G G V V K N N F V P T N V G S K A F
4) S C N T A T C V T H R L A G L L S R S G G V V K D N F V P T N V G S E A F
```

It is known that CGRP exerts significant effects on blood pressure and blood catecholamine levels when administered to rats.

In an article in the Lancet published on 2 August 1987, A. Clark, G.J.S. Cooper et al. report that islet amyloid in twenty-two amyloid-containing type 2 diabetic subjects showed immunoreactivity with antisera to CGRP. This was inhibited by preabsorption of the antisera with amylin, which suggests that amylin is a major protein constituent of islet amyloid. Cooper et al., supra (1987), confirmed that amylin is a major component of islet amyloid. In addition CGRP amylin immunoreactivity was found in islet cells of both diabetic and non-diabetic subjects, and preliminary studies show its presence in B-cells. Cooper et al., Lancet 1987, 2:964. This identification, together with the finding of a similar peptide in insulinomas, suggests that amylin may be cosecreted with insulin.

Carboxy terminally amidated amylin or a functional peptide fragment thereof or a conservative variant of the carboxy terminally amidated amylin or fragment will be of use in the treatment of diabetes mellitus or hypoglycaemia. This idea was unexpected. Although it was known that both amylin and CGRP are associated in some way with diabetes mellitus, it had not previously been suggested that either might be useful in the treatment of the condition.

The structure of amylin (in its amidated form) may be represented as:-

$$R\text{-}NH\text{-}(C_5H_4OH)\text{-}CONH_2$$

where R is the residue of the peptide up to the peptide bond to the carboxy terminal residue Tyrosine.

The invention provides a synthetic peptide having the amino acid sequence:

KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY

said peptide being carboxy terminally amidated and having a disulphide bond. The dissulphide bond is formed between the cysteine residues at positions 2 and 7.

The invention also provides a synthetic, carboxy terminally amidated peptide which is a conservative variant of the above peptide.

By a conservative variant is meant a peptide which is substantially, though not completely, homologous with amylin but which is functionally equivalent thereto. (See M.O. Dayhoff, A Model of Evolutionary Change in Proteins, in "Atlas of Protein Sequence and Structure", volume 5, supplement 3, National Biomedical Research Foundation, 1978, pages 345 to 352).

According to a preferred aspect of the invention, a composition for use in the treatment of diabetes mellitus or hypoglycaemia comprises a) insulin and b) carboxy terminally amidated amylin or a functional peptide fragment thereof or of a conservative variant of the carboxy terminally amidated amylin or fragment. The term insulin is here used to cover insulin of natural and synthetic origin and also functional peptide fragments of insulin and conservative variants of insulin or fragments thereof such as may be used in the conventional treatment of diabetes mellitus.

Products according to the invention may conveniently be provided in the form of solutions suitable for parenteral administration. In many cases, it will be convenient to provide insulin and amylin (or variant) in a single solution for administration together. In other cases, it may be more advantageous to administer insulin, and amylin (or variant), separately. A suitable administration regime may best be determined by a doctor for each patient individually. It will generally be preferable to formulate such that the molar ratio of insulin to amylin (or variant) used for the treatment is from 100:1 to 0.1:1. A preliminary study has indicated that, like insulin immunoreativity, amylin immunoreactivity and hence amylin, is absent from the islet of Langerhans in type 1 diabetics. It is therefore proposed that the type 1 diabetic syndrome results from a deficiency of not one (i.e. insulin as previously thought) but two (insulin and amylin) hormones. As previously noted, the major problem with insulin treatment of diabetes is hypoglycaemia. It is likely that co-administration of insulin and amylin may avoid this side effect. This may then allow:-

- Tighter diabetic control with reduced risk of hypoglycaemia. This applies to the treatment of type 1 diabetes mellitus, and also for type 2 diabetes mellitus (in the phrase of secondary islet cell failure).

- The use of amylin for the therapy of recurrent hypoglycaemia complicating the insulin therapy of type 1 diabetes mellitus (or of type 2 diabetes mellitus).
- The therapy of brittle diabetes (type 1 diabetes mellitus with increased risk of hypoglycaemia).
- The therapy of the intractable hypoglycaemia which may complicate the course of the disease produced by insulin secreting tumours, such as insulinomas.

Although this invention is concerned with results and not with theories, the following explanation of the possible mode of action of amylin may be of interest.

1. Amylin is produced in the islets of Langerhans, almost certainly in the B-cell i.e. the same cell that produces insulin. Type 1 diabetes results from the destruction of B-cells in the islets of Langerhans As these cells contain amylin then it is very likely that type 1 diabetes is associated with a deficiency of amylin as well as insulin. Certainly, amylin is not seen in the islets of Langerhans in this condition.

2. Amylin and CGRP have been shown to modulate the rate of glucose induced insulin secretion from islet B cells in a number of model systems. (Ahren B. Martensson H. Nobin A. Effects of calcitonin gene related peptide (CGRP) on islet hormone secretion in the pig. Diabetologia 1987; 30: 354-359).

3. In isolated rat soleus muscles, amylin reduces the rate of glycogen synthesis in both the basal and the insulin-stimulated modes (see Example below).

When 2 and 3 are taken together, amylin (or CGRP) exerts a powerful modulating effect on insulin-induced storage of glucose as glycogen. As this may well be the mechanism whereby insulin resistance is caused in type 2 diabetes, then it may well be that hypersecretion of amylin (or CGRP) is a factor in the genesis of the insulin resistance found in that condition.

The actions of amylin or (CGRP) as seen above, modulate and reduce the hypoglycaemic effects of insulin, both by reducing the release of insulin in relation to a given glucose stimulus, and (more importantly in the case of type 1 diabetes) by reducing the rate of storage of glucose as glycogen. Hence, amylin (or CGRP) may include "insulin resistance", and cushion the hypoglycaemic effects of insulin.

The efficacy of a preparation of amylin in the treatment of diabetes mellitus is dependent on the ability of the amylin to gain access to the circulation. To that end, preparations of amylin that are soluble are required. It has been demonstrated that certain processes may be used to solubilize amylin when present in amyloid masses, and these methods will also be of use in solubilizing amylin from other sources. Cooper et al., Proc. Natl. Acad. Sci. USA. 84:8628-8632 (1987). They include (1) dissolution of amylin in guanidinium solutions, especially guanidinium hydrochloride, pH 7.5, buffered in 0.2 M sodium monohydrogen phosphate/sodium dihydrogen phosphate; (2) dissolution of amylin in trifluoroacetic acid acetonitrile solutions, especially 1.0% trifluoroacetic acid 67% acetonitrile; (3) dissolution of amylin in formic acid solution, especially 70% formic acid; and (4) the use of the ultrasound to dissolve amylin.

Experimental work also indicates that lyophilization may render amylin more soluble, perhaps by altering its physical state.

Comparison of the activity of amylin which has been chemically synthesized with that from natural sources indicates that the activity of amylin from these differing sources can be quantitatively different. It is likely that any difference in activity is caused by a lack during chemical synthesis to completely reconstitute the molecule into the natural conformation necessary for full biological activity. This is believed caused in part by failure to completely reconstitute the disulphide bond in the chemically synthesized material. Therefore, it is useful to observe that the activity of amylin from different sources i.e., extracted from the natural state, and chemically synthesized, may be different owing in part to differing degrees of reconstitution of the natural secondary and tertiary structure of the molecule.

Reconstruction of the molecule in dilute aqueous solution at pH8 produced a degree of biological activity. In view of the low solubility of the synthetic material in solution in pH 8 water, however, improved reconstitution of synthetic amylin may be obtained by refolding material resulting from synthetic methods, in an aqueous denaturing solution, for example, guanidinium or urea solutions, especially 6.0 M guanidinium chloride or 8.0 urea, at a specified pH, especially a mildly alkaline pH between about 7.5 and about 9.0. Alternatively, solution for subsequent reconstitution may be effective in a non-aqueous, denaturing solvent, such as dimethyl formamide. Under such conditions, the reconstruction of the disulphide bond by mild oxidation, such as produced by solutions of potassium, ferricyanide or by exposure to atmospheric oxygen, are expected to produce optimal reconstitution of the disulphide bond.

For therapeutic use, it will be useful to have amylin preparations of differing durations of action, such as those in use for insulin. See Larner. J., "Insulin and Oral Hypogylcaemic Drugs; Glucagon". (In Gilman, et al., Eds. The Pharmacologic Basis of Therapeutics, 7th Edition, MacMillan 1985, p. 1501-02). To that end, methods similar to those used for

insulin are to be employed for therapeutic preparations of amylin. All such preparations may be used either alone, or in combination with appropriate combinations of insulin, for the treatment of diabetes mellitus, hypoglycaemia and other conditions. These procedures and preparations include (1) reaction of amylin with zinc and protamine, according to the method of Hagedorn, et al., "Protamine Insulinate," JAMA 106:177-180 (1936), to produce an amylin preparation, the onset and duration of action of which is delayed compared with that of non-complexed amylin; (2) a suspension of the protamine-amylin prepared as above in a suitable aqueous buffer for parenteral administration; (3) crystalline amylin prepared by the crystallization of amylin in the presence of a zinc salt, especially zinc chloride in a suitable buffer medium, and especially one of neutral pH (Larner, supra); (4) a suspension of crystalline zinc amylin in a suitable aqueous buffer, prepared as above, and suitable for parenteral administration; (5) a modified protamine zinc suspension of amylin that is crystalline where the concentrations of amylin, zinc and protamine may be so arranged that the onset and duration of action are intermediate between those of the soluble and protamine forms of amylin; (6) the material of (5) above formulated in a suitable aqueous buffer useful for parenteral administration; (7) a preparation of crystalline zinc-amylin resuspended in a solution of sodium chloride/sodium acetate, pH 7.2-7.5, and suitable for parenteral administration; (8) amorphous insulin precipitated at high pH and suitable for parenteral administration; and (9) a mixture of crystalline and amorphous amylin suitable for parenteral administration. Each such preparation will be suitable for parenteral administration by the subcutaneous route.

The stability of amylin preparations may be increased at neutral pH. Netutral preparations of amylin may be mixed with other neutral preparations of amylin, or with appropriate preparations of insulin resulting in increased clinical utility. Larner, supra.

To purify amylin from various different sources to a level useful in human therapeutics, various methods have been used. It has been demonstrated that amylin can be isolated from the human pancreas in a highly pure state by a combination of concentration using a centrifuge, gel filtration chromatography, and reverse phase chromatography, specifically HPLC. In, for example, larger scale purification of amylin, forms of chromatography other than HPLC, such as fast protein liquid chromatography (FPLC), may be useful and appropriate. It is also possible that other forms of chromatography may be useful, such as ion exchange, molecular sieve, or hydrophobic interaction chromatography.

Therapy of Type 1 diabetes mellitus with transplants of islet cell tissue or whole pancreatic tissues, or with islet cell implants may well become important treatments. Because some of the therapeutic effects of such therapy will accrue from replacement of the ability to manufacture and secrete amylin, monitoring of amylin levels will be necessary to follow the course of such therapy. Similarly, it may be necessary to monitor amylin levels in blood, serum or plasma, to assess the treatment of hypoglycaemia, Type 1 diabetes mellitus, and various amylin-deficient states.

EXAMPLE

This experiment was performed to demonstrate 3 above, that amylin reduces the rate of glycogen synthesis in both basal and insulin-stimulated modes.

After having been starved overnight, rats were killed and their soleus muscles extracted and incubated in buffer at physiological pH. ^{14}C-labelled glucose and cold (unlabelled) glucose were added and the rate of incorporation of glucose into glycogen was measured by extraction of glycogen and counting at intervals of up to six hours. The experiments were done in the presence, 1, 10, 100 and 1000 microunits of insulin per ml. Half the experiments were performed in the presence of 120 nanomoles per litre of amylin.

The results are set out in the accompanying Figure 1, which is a graph of rate of glycogen synthesis against insulin concentration. The open circles represent the results of experiments performed in the absence of amylin; the filled circles represent results of experiments performed in the presence of 15 micromoles per litre of amylin. Each spot at 1 and 100 microunits per ml insulin is the mean of 11 replicate experiments; each spot at 10 and 1000 microunits per ml insulin is the mean of 5 replicates.

At all physiological concentrations of insulin (from 1 to 100 microunits per ml), glycogen synthesis is slowed down in the presence of amylin. The differences are statistically significant (p is less than 0.05 at 1 and 100 microunits per ml by the Mann Whitney U test.

It will be observed that the inhibition of glycogen synthesis by amylin persists at low, and presumably even at zero insulin concentrations. It appears that amylin has its own action which is contrary to that of insulin but probably not mediated by direct antagonism of insulin action. In support of this, it has been observed that amylin is not capable of significantly displacing insulin from its receptor on red blood cells.

Claims

1. A synthetic peptide having the amino acid sequence:
   KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY
   said peptide being carboxy terminally amidated and having a disulphide bond between the cysteine residues at positions 2 and 7.

2. A synthetic carboxy terminally amidated peptide which is a conservative variant of a peptide of claim 1.

3. A method of enhancing the biological activity of a carboxy terminally amidated peptide having the sequence defined in claim 1, comprising refolding said peptide in a denaturing solution.

4. A method as claimed in claim 3 wherein said denaturing solution is a guanidinium or urea solution.

5. A method as claimed in claim 4 wherein said denaturing solution is at a pH of between about 7.5 and about 9.0.

6. A method as claimed in any of claims 3 to 5 wherein a disulphide bond is formed by mild oxidation.

7. A method of purifying a carboxy terminally amidated peptide having the amino acid sequence defined in claim 1, the method comprising subjecting the peptide to a form of chromatography other than reverse phase chromatography.

8. A method according to claim 7 wherein the peptide is subjected to fast protein liquid chromatography, ion exchange chromatography, molecular sieve chromatography or hydrophobic interaction chromatography.

9. A composition for use in therapy comprising a synthetic peptide as claimed in claim 1 or claim 2 or a peptide obtainable by a method as claimed in any of claims 3 to 8, the peptide having been treated to provide a delayed release preparation.

10. A composition as claimed in claim 9 which is formulated with protamine.

11. A composition as claimed in claim 9 or claim 10 which is formulated with a zinc salt.

12. A composition as claimed in claim 11 wherein said peptide is in crystalline form.

13. A composition as claimed in claim 11 or claim 12 wherein said zinc salt is zinc chloride.

14. A composition as claimed in any of claims 9 to 13 which is suitable for parenteral administration.

15. A composition according to any one of claims 9 to 14 wherein the disulphide bond is intact.

16. A composition according to any of claims 9 to 14 wherein the disulphide bond is not intact.

17. A pharmaceutical product comprising a synthetic peptide as claimed in claim 1 or claim 2, or a peptide obtainable by a method as claimed in any of claims 3 to 8, and an insulin, as a combined preparation for use separately or together in treatment by therapy.

18. Use of a synthetic peptide as claimed in claim 1 or claim 2 in the preparation of a medicament for treating a subject for diabetes mellitus.

19. The use as claimed in claim 18 wherein insulin is used in the preparation of said medicament.

20. The use as claimed in claim 18 or claim 19 wherein said synthetic peptide is formulated for parenteral administration.

21. The use as claimed in any of claims 12 to 14 wherein said synthetic peptide is formulated for delayed release administration.

22. A peptide obtainable from the islet amyloid of diabetic pancreata comprising the steps of:

(a) solubilising said amyloid,
(b) subjecting the amyloid material from step (a) to normal phase high performance liquid chromatography gel filtration, and
(c) subjecting the amyloid material of step (b) to reverse phase high performance liquid chromatography followed by elution.

23. A peptide obtainable from the islet amyloid of diabetic pancreata by a process comprising the steps of (a) using formic acid in conjunction with ultrasound to solubilise amyloid material obtained from such pancreata (b) subjecting the amyloid material from step (a) to normal phase high performance liquid chromatography gel filtration using a mobile phase comprising aqueous guanidine and sodium phosphate, and (c) subjecting the amyloid material from step (b) to high performance liquid chromatography using a mobile phase comprising trifuoroacetic acid and elution by acetonitrile.

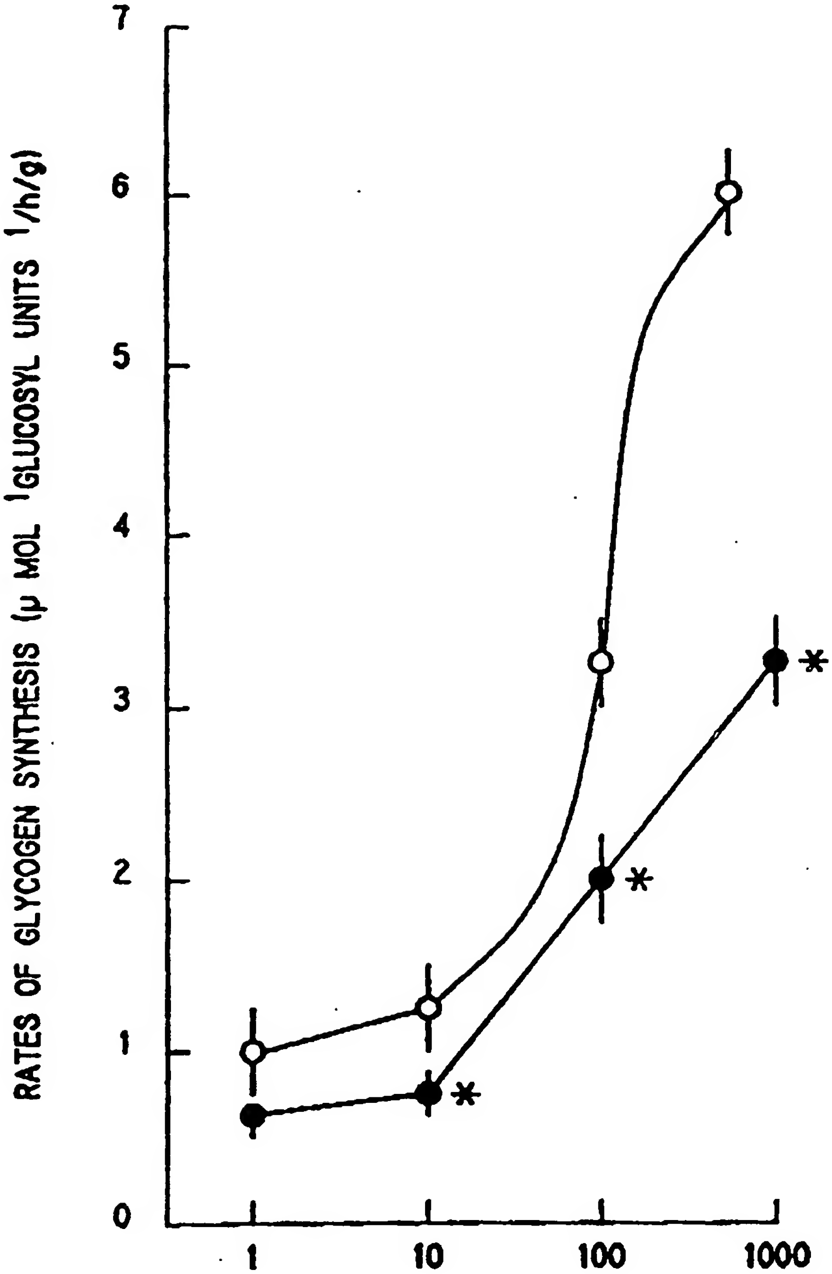
RATES OF GLYCOGEN SYNTHESIS (μ MOL ¹GLUCOSYL UNITS ¹/h/g)
0
1
2
3
4
5
6
7
1
10
100
1000
*
*
*

FIG. 1.

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EUROPEAN SEARCH REPORT

Application Number
EP 96 11 9980

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	BIOCHEM. BIOPHYS. RES. COMMUN. (1986), 140(3), 827-31 CODEN: BBRCA9;ISSN: 0006-291X, 14 November 1986, XP000673997 WESTERMARK, PER ET AL: "A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas" * page 828, paragraph 1; figure 2 * ---	1,3,4,7, 8,15,16, 22,23	C07K14/575 A61K38/22 A61K38/28 //(A61K38/28, 38:22)
X	AM. J. PATHOL. (1987), 127(3), 414-17 CODEN: AJPAA4;ISSN: 0002-9440, June 1987, XP000673916 WESTERMARK, PER ET AL: "Islet amyloid in type 2 human diabetes mellitus and adult diabetic cats contains a novel putative polypeptide hormone" * page 415, left-hand column, paragraph 3 - right-hand column, paragraph 1; figure 2 * * page 416, right-hand column, paragraph 1 - page 417, left-hand column, paragraph 3 * ---	1,3,4,7, 8,15,16, 22,23	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07K A61K
X	ENDOCRINOLOGY, vol. 119, no. 2, July 1986, pages 865-869, XP000673903 M. PETTERSSON ET AL.: "Calcitonin Gene-Related Peptide: Occurence in Pancreatic Islets in the Mouse and the Rat and Inhibition of Insulin Secretion in the Mouse" * page 386 - page 387 * ---	1,3,4,7, 8,15,16, 22,23	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	29 May 1997	Fuhr, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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D,X	LANCET THE, vol. II/1987, no. 8553, 1 August 1987, LONDON GB, pages 231-234, XP002031852 A. CLARK ET AL.: "Islet Amyloid Formed from Diabetes-Associated Peptide may be Pathogenic in Type-2 Diabetes" * page 232, left-hand column, paragraph 1; figure 1 * * page 233, left-hand column, paragraph 1 - page 234, left-hand column, paragraph 1 *	1,3,4,7, 8,15,16, 22,23	
D,P, X	PROC. NATL. ACAD. SCI. U. S. A. (1987), 84(23), 8628-32 CODEN: PNASA6;ISSN: 0027-8424, December 1987, XP002031853 COOPER, G. J. S. ET AL: "Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients" * page 8628, right-hand column, paragraph 3 - page 8629, right-hand column, paragraph 2 * * page 8630, right-hand column, paragraph 4 - page 8631, right-hand column, paragraph 2; figure 2 *	1,3,4,7, 8,15,16, 22,23	
P,X	EP 0 263 802 A (WESTERMARK PER ;UNIV MINNESOTA (US)) 13 April 1988 * claims; examples *	1,3,4,7, 8,15,16, 22,23	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,E	EP 0 289 287 A (AMYLIN CORP) 2 November 1988 * page 4, line 36 - line 41 * * page 5, line 60 - page 6, line 4; claims; examples * ---	1-4,6-8, 15,16, 18,22,23	
A	EP 0 191 349 A (GAMBRO LUNDIA AB) 20 August 1986 * the whole document * -----	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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